



## SEQUENCE LISTING

<110> Shyur, Lie-Fen  
Chen, Jui-Lin  
Yang, Ning-Sun

<120> A Truncated Form of Fibrobacter Succinogenes 1,3-1,4-Beta-D-Glucanase With Improved

Enzymatic Activity And Thermo-Tolerance

<130> 4910-8

<140> US 09/654,652

<141> 2000-09-05

<150>

<151>

<160> 6

<210> 1

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified enzyme with enhanced activity and thermal stability

<400> 1

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr

|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| 1   | 5   | 10  | 15  | 20  |
| Gly | Lys | Phe | Glu | Ala |
| Arg | Met | Lys | Met | Ala |
| Ala | Ala | Ala | Ser | Gly |
| Thr | Val | Ser | Ser | Met |
| Phe | 25  | 30  | 35  | 40  |
| Leu | Tyr | Gln | Asn | Gly |
| Ser | Glu | Ile | Ala | Asp |
| Gly | Arg | Pro | Trp | Val |
| Glu | Val | Asp | Ile | Glu |
| 60  | 45  | 50  | 55  | 60  |
| Val | Leu | Gly | Lys | Asn |
| Pro | Gly | Ser | Phe | Gln |
| Ser | Asn | Ile | Ile | Thr |
| Gly | Lys | Ala | Gly | Ala |
| 80  | 65  | 70  | 75  | 80  |
| Gln | Lys | Thr | Ser | Glu |
| Lys | His | His | Ala | Val |
| Ser | Pro | Ala | Ala | Asp |
| Gln | Ala | Phe | His | Thr |
| 100 | 85  | 90  | 95  | 100 |
| Tyr | Gly | Leu | Glu | Trp |
| Thr | Pro | Asn | Tyr | Val |
| Arg | Trp | Thr | Val | Asp |
| Gly | Gln | Glu | Val | Arg |
| 120 | 105 | 110 | 115 | 120 |
| Lys | Thr | Glu | Gly | Gln |
| Val | Ser | Asn | Leu | Thr |
| Gly | Thr | Gln | Gly | Leu |
| Arg | Phe | Asn | Leu | 140 |
| 145 | 125 | 130 | 135 | 140 |
| Trp | Ser | Ser | Glu | Ser |
| Ala | Ala | Trp | Val | Gly |
| Gln | Phe | Asp | Glu | Ser |
| Lys | Leu | Pro | Leu | 160 |
| 165 | 145 | 150 | 155 | 160 |
| Gln | Phe | Ile | Asn | Trp |
| Val | Lys | Val | Tyr | Lys |
| Tyr | Thr | Pro | Gly | Gln |
| Gly | Glu | Gly | Gly | 180 |
| 185 | 165 | 170 | 175 | 180 |
| Asp | Phe | Thr | Leu | Asp |
| Trp | Thr | Asp | Asn | Phe |
| Asp | Thr | Phe | Asp | Gly |
| Ser | Arg | Trp | Gly | 200 |
| 205 | 185 | 190 | 195 | 200 |
| Gly | Asp | Trp | Thr | Phe |
| Asp | Gly | Asn | Arg | Val |
| Asp | Leu | Thr | Asp | Lys |
| Asn | Ile | Tyr | Ser | Arg |
| 220 | 205 | 210 | 215 | 220 |
| Asp | Gly | Met | Leu | Ile |
| Leu | Ala | Leu | Thr | Arg |
| Lys | Gly | Gln | Glu | Ser |
| Phe | Asn | Gly | Gln | 240 |
| 225 | 230 | 235 |     | 240 |
| Pro | Arg | Asp | Asp | Glu |
| Pro | Ala | Pro |     | 245 |

<210> 2

<211> 267

<212> PRT

<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; Modified enzyme with enhanced activity and thermal stability

&lt;400&gt; 2

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| Met | Val | Ser | Ala | Lys | Asp | Phe | Ser | Gly | Ala | Glu | Leu | Tyr | Thr | Leu | Glu | Glu | Val | Gln | Tyr | 1   | 5   | 10  | 15  | 20 |
| Gly | Lys | Phe | Glu | Ala | Arg | Met | Lys | Met | Ala | Ala | Ala | Ser | Gly | Thr | Val | Ser | Ser | Met | Phe | 25  | 30  | 35  | 40  |    |
| Leu | Tyr | Gln | Asn | Gly | Ser | Glu | Ile | Ala | Asp | Gly | Arg | Pro | Trp | Val | Glu | Val | Asp | Ile | Glu | 45  | 50  | 55  | 60  |    |
| Val | Leu | Gly | Lys | Asn | Pro | Gly | Ser | Phe | Gln | Ser | Asn | Ile | Ile | Thr | Gly | Lys | Ala | Gly | Ala | 65  | 70  | 75  | 80  |    |
| Gln | Lys | Thr | Ser | Glu | Lys | His | His | Ala | Val | Ser | Pro | Ala | Ala | Asp | Gln | Ala | Phe | His | Thr | 85  | 90  | 95  | 100 |    |
| Tyr | Gly | Leu | Glu | Trp | Thr | Pro | Asn | Tyr | Val | Arg | Trp | Thr | Val | Asp | Gly | Gln | Glu | Val | Arg | 105 | 110 | 115 | 120 |    |
| Lys | Thr | Glu | Gly | Gly | Gln | Val | Ser | Asn | Leu | Thr | Gly | Thr | Gln | Gly | Leu | Arg | Phe | Asn | Leu | 125 | 130 | 135 | 140 |    |
| Trp | Ser | Ser | Glu | Ser | Ala | Ala | Trp | Val | Gly | Gln | Phe | Asp | Glu | Ser | Lys | Leu | Pro | Leu | Phe | 145 | 150 | 155 | 160 |    |
| Gln | Phe | Ile | Asn | Trp | Val | Lys | Val | Tyr | Lys | Tyr | Thr | Pro | Gly | Gln | Gly | Glu | Gly | Gly | Ser | 165 | 170 | 175 | 180 |    |
| Asp | Phe | Thr | Leu | Asp | Trp | Thr | Asp | Asn | Phe | Asp | Thr | Phe | Asp | Gly | Ser | Arg | Trp | Gly | Lys | 185 | 190 | 195 | 200 |    |
| Gly | Asp | Trp | Thr | Phe | Asp | Gly | Asn | Arg | Val | Asp | Leu | Thr | Asp | Lys | Asn | Ile | Tyr | Ser | Arg | 205 | 210 | 215 | 220 |    |
| Asp | Gly | Met | Leu | Ile | Leu | Ala | Leu | Thr | Arg | Lys | Gly | Gln | Glu | Ser | Phe | Asn | Gly | Gln | Val | 225 | 230 | 235 | 240 |    |
| Pro | Arg | Asp | Asp | Glu | Pro | Ala | Pro | Asn | Ser | Ser | Ser | Val | Asp | Lys | Leu | Ala | Ala | Ala | Leu | 245 | 250 | 255 | 260 |    |
| Glu | His | His | His | His | His | His | His |     |     |     |     |     |     |     |     |     |     |     |     | 265 |     |     |     |    |

&lt;210&gt; 3

&lt;211&gt; 349

&lt;212&gt; PRT

&lt;213&gt; Fibrobacter succinogenes

&lt;220&gt;

&lt;223&gt;

&lt;400&gt; 3

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| Met | Asn | Ile | Lys | Lys | Thr | Ala | Val | Lys | Ser | Ala | Leu | Ala | Val | Ala | Ala | Ala | Ala | Ala | Ala | 1   | 5   | 10  | 15  | 20 |
| Leu | Thr | Thr | Asn | Val | Ser | Ala | Lys | Asp | Phe | Ser | Gly | Ala | Glu | Leu | Tyr | Thr | Leu | Glu | Glu | 25  | 30  | 35  | 40  |    |
| Val | Gln | Tyr | Gly | Lys | Phe | Glu | Ala | Arg | Met | Lys | Met | Ala | Ala | Ala | Ser | Gly | Thr | Val | Ser | 45  | 50  | 55  | 60  |    |
| Ser | Met | Phe | Leu | Tyr | Gln | Asn | Gly | Ser | Glu | Ile | Ala | Asp | Gly | Arg | Pro | Trp | Val | Glu | Val | 65  | 70  | 75  | 80  |    |
| Asp | Ile | Glu | Val | Leu | Gly | Lys | Asn | Pro | Gly | Ser | Phe | Gln | Ser | Asn | Ile | Ile | Thr | Gly | Lys | 85  | 90  | 95  | 100 |    |
| Ala | Gly | Ala | Gln | Lys | Thr | Ser | Glu | Lys | His | His | Ala | Val | Ser | Pro | Ala | Ala | Asp | Gln | Ala | 105 | 110 | 115 | 120 |    |
| Phe | His | Thr | Tyr | Gly | Leu | Glu | Trp | Thr | Pro | Asn | Tyr | Val | Arg | Trp | Thr | Val | Asp | Gly | Gln | 125 | 130 | 135 | 140 |    |
| Glu | Val | Arg | Lys | Thr | Glu | Gly | Gly | Gln | Val | Ser | Asn | Leu | Thr | Gly | Thr | Gln | Gly | Leu | Arg | 145 | 150 | 155 | 160 |    |
| Phe | Asn | Leu | Trp | Ser | Ser | Glu | Ser | Ala | Ala | Trp | Val | Gly | Gln | Phe | Asp | Glu | Ser | Lys | Leu | 165 | 170 | 175 | 180 |    |
| Pro | Leu | Phe | Gln | Phe | Ile | Asn | Trp | Val | Lys | Val | Tyr | Lys | Tyr | Thr | Pro | Gly | Gln | Gly | Glu | 185 | 190 | 195 | 200 |    |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Ser | Asp | Phe | Thr | Leu | Asp | Trp | Thr | Asp | Asn | Phe | Asp | Thr | Phe | Asp | Gly | Ser | Arg |
|     |     |     |     | 205 |     |     |     |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |
| Trp | Gly | Lys | Gly | Asp | Trp | Thr | Phe | Asp | Gly | Asn | Arg | Val | Asp | Leu | Thr | Asp | Lys | Asn | Ile |
|     |     |     |     | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Tyr | Ser | Arg | Asp | Gly | Met | Leu | Ile | Leu | Ala | Leu | Thr | Arg | Lys | Gly | Gln | Glu | Ser | Phe | Asn |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |     |     | 260 |
| Gly | Gln | Val | Pro | Arg | Asp | Asp | Glu | Pro | Ala | Pro | Gln | Ser | Ser | Ser | Ser | Ala | Pro | Ala | Ser |
|     |     |     |     | 265 |     |     |     |     | 270 |     |     |     |     | 275 |     |     |     |     | 280 |
| Ser | Ser | Ser | Val | Pro | Ala | Ser | Ser | Ser | Ser | Val | Pro | Ala | Ser | Ser | Ser | Ser | Ala | Phe | Val |
|     |     |     |     | 285 |     |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |
| Pro | Pro | Ser | Ser | Ser | Ser | Ala | Thr | Asn | Ala | Ile | His | Gly | Met | Arg | Thr | Thr | Pro | Ala | Val |
|     |     |     |     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Ala | Lys | Glu | His | Arg | Asn | Leu | Val | Asn | Ala | Lys | Gly | Ala | Lys | Val | Asn | Pro | Asn | Gly | His |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |     |     |     | 340 |
| Lys | Arg | Tyr | Arg | Val | Asn | Phe | Glu | His |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 345 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

<210> 4  
 <211> 744  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> Code for a modified enzyme  
 <400> 4

|            |            |             |             |            |             |     |
|------------|------------|-------------|-------------|------------|-------------|-----|
| atggttagcg | caaaggattt | tagcgggtgcc | gaactctaca  | cgttagaaga | agttcagtag  | 60  |
| ggtaagtttg | aagcccgtat | gaagatggca  | gccgcacgcg  | gaacagtcag | ttccatgttc  | 120 |
| ctctaccaga | atggttccga | aatcgccgat  | ggaaggccct  | gggtagaagt | ggatattgaa  | 180 |
| gttctcggca | agaatccggg | cagtttccag  | tccaacatca  | ttaccggtaa | ggccggcgca  | 240 |
| caaaagacta | gcgaaaagca | ccatgctgtt  | agccccgcgc  | ccgatcaggc | tttccacacc  | 300 |
| tacggtctcg | aatggactcc | gaattacgtc  | cgctggactg  | ttgacggtca | ggaagtccgc  | 360 |
| aagacggaag | gtggccaggt | ttccaacttg  | acaggtacac  | agggactccg | ttttaacctt  | 420 |
| tggtcgtctg | agagtgcggc | ttgggttggc  | cagttcgtatg | aatcaaagct | tccgcttttc  | 480 |
| cagttcatca | actgggtcaa | ggtttataag  | tatacgccgc  | gccagggcga | aggcggcagc  | 540 |
| gactttacgc | ttgactggac | cgacaatttt  | gacacgtttg  | atggctcccg | ctggggcaag  | 600 |
| ggtgactgga | catttgacgg | taaccgtgtc  | gacctcaccg  | acaagaacat | ctactccaga  | 660 |
| gatggcatgt | tgatcctcgc | cctcaccgcg  | aaaggtcagg  | aaagcttcaa | cggccagggtt | 720 |
| ccgagagatg | acgaacctgc | tccg        |             |            |             | 744 |

<210> 5  
 <211> 804  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> Code for a modified enzyme  
 <400> 5

|            |            |             |             |            |             |     |
|------------|------------|-------------|-------------|------------|-------------|-----|
| atggttagcg | caaaggattt | tagcgggtgcc | gaactctaca  | cgttagaaga | agttcagtag  | 60  |
| ggtaagtttg | aagcccgtat | gaagatggca  | gccgcacgcg  | gaacagtcag | ttccatgttc  | 120 |
| ctctaccaga | atggttccga | aatcgccgat  | ggaaggccct  | gggtagaagt | ggatattgaa  | 180 |
| gttctcggca | agaatccggg | cagtttccag  | tccaacatca  | ttaccggtaa | ggccggcgca  | 240 |
| caaaagacta | gcgaaaagca | ccatgctgtt  | agccccgcgc  | ccgatcaggc | tttccacacc  | 300 |
| tacggtctcg | aatggactcc | gaattacgtc  | cgctggactg  | ttgacggtca | ggaagtccgc  | 360 |
| aagacggaag | gtggccaggt | ttccaacttg  | acaggtacac  | agggactccg | ttttaacctt  | 420 |
| tggtcgtctg | agagtgcggc | ttgggttggc  | cagttcgtatg | aatcaaagct | tccgcttttc  | 480 |
| cagttcatca | actgggtcaa | ggtttataag  | tatacgccgc  | gccagggcga | aggcggcagc  | 540 |
| gactttacgc | ttgactggac | cgacaatttt  | gacacgtttg  | atggctcccg | ctggggcaag  | 600 |
| ggtgactgga | catttgacgg | taaccgtgtc  | gacctcaccg  | acaagaacat | ctactccaga  | 660 |
| gatggcatgt | tgatcctcgc | cctcaccgcg  | aaaggtcagg  | aaagcttcaa | cggccagggtt | 720 |

ccgagagatg acgaacctgc tccgaattcg agctccgtcg acaagcttgc ggccgcactc 780  
 gagcaccacc accaccacca ctga 804

<210> 6  
 <211> 1050  
 <212> DNA  
 <213> Fibrobacter succinogenes  
 <220>  
 <223>  
 <400> 6

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| atgaacatca | agaaaactgc | agtcaagagc | gctctcgccg | tagcagccgc | agcagcagcc | 60   |
| ctcaccacca | atgttagcgc | aaaggatttt | agcggtgccg | aactctacac | gttagaagaa | 120  |
| gttcagtacg | gtaagtttga | agcccgtatg | aagatggcag | ccgcatcggg | aacagtcagt | 180  |
| tccatgttcc | tctaccagaa | tggttccgaa | atcgccgatg | gaaggccctg | ggtagaagtg | 240  |
| gatatagaag | ttctcggcaa | gaatccgggc | agtttccagt | ccaacatcat | taccggtaag | 300  |
| gccggcgcac | aaaagactag | cgaaaagcac | catgctgtta | gccccgcgcg | cgatcaggct | 360  |
| ttccacacct | acggtctcga | atggactccg | aattacgtcc | gctggactgt | tgacggtcag | 420  |
| gaagtccgca | agacggaagg | tggccagggt | tccaacttga | caggtagaca | gggactccgt | 480  |
| tttaaccttt | ggtcgtctga | gagtgcggct | tgggttggcc | agttcgatga | atcaaagctt | 540  |
| ccgcttttcc | agttcatcaa | ctgggtcaag | gtttataagt | atacgccggg | ccagggcgaa | 600  |
| ggcggcgacg | actttacgct | tgactggacc | gacaattttg | acacgtttga | tggtccccgc | 660  |
| tggggcaagg | gtgactggac | atttgacggt | aaccgtgtcg | acctcaccga | caagaacatc | 720  |
| tactccagag | atggcatggt | gacccctgcc | ctcaccgcga | aaggtcagga | aagcttcaac | 780  |
| ggccagggtc | cgagagatga | cgaacctgct | ccgcaatctt | ctagcagcgc | tccggcatct | 840  |
| tctagcagtg | ttccggcaag | ctcctctagc | gtccctgcct | cctcgagcag | cgatttggtt | 900  |
| ccgccgagct | cctcgagcgc | cacaaacgca | atccacggaa | tgcgcacaac | tccggcagtt | 960  |
| gcaaaggaac | accgcaatct | cgtgaacgcc | aagggtgcc  | aggtgaacct | gaatggccac | 1020 |
| aagcgttatc | gcgtgaactt | tgaacactaa |            |            |            | 1050 |

<210> 7  
 <211> 13  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> PCR primer  
 <400> 7

tcaccaccat ggttagcgca aag 13

<210> 8  
 <211> 15  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> PCR primer  
 <400> 8

gccacgaatt ctgttcaaag ttcac 15

<210> 9  
 <211> 17  
 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> PCR Primer  
 <400> 9

cagccggcga tggccatggt tagcgca 17

<210> 10  
<211> 19  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> PCR Primer  
<400> 10  
ctgctagaag aattcggagc aggttcgtc

19

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